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Antioxidative properties of Nauclea latifolia (African peach) extracts protects against sodium arsenite-induced oxidative damage in hepatorenal tissues in Wistar rats

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Abstract

Background Food and water serve as the primary sources of arsenic exposure, posing one of the most significant health threats related to heavy metals. Arsenic causes oxidative stress, which has many negative effects on humans. *Nauclea latifolia* (African peach) stem bark contains bioactive compounds with demonstrated antioxidant and free radical scavenging properties that may offer protection against heavy metal toxicity. This study investigated the protective effects of *N. latifolia* stem bark aqueous extract against sodium arsenite-induced oxidative damage in hepatorenal tissues. A total of 25 rats were separated into five groups, each containing five rats. Each rat received the prescribed treatment once daily for 28 days. A control group received no $NaAsO_2$, while a second group received 5 mg/kg $NaAsO_2$. The treatment groups received *N. latifolia* stem bark aqueous extracts (200 and 400 mg/kg) and silymarin (50 mg/kg), followed by 5 mg/kg of $NaAsO_2$.

Results Rats administered $NaAsO_2$ showed a significant decrease ($p < 0.05$) in SOD, CAT, and GSH levels, but significant increases ($p < 0.05$) in proinflammatory marker (TNF-α & IL-6) levels, urea, creatinine, and liver enzyme biomarkers. Treatment with *N. latifolia* stem bark aqueous extract and silymarin ameliorated oxidative stress and inflammation, and reduced levels of creatinine, uric acid, urea and liver enzyme activity.

Conclusion The *N. latifolia* stem bark aqueous extract prevented histological hepatic steatosis and renal cytoarchitecture deterioration induced by $NaAsO_2$ exposure. This suggests that plant extracts with antioxidant compounds like *N. latifolia* can mitigate liver and kidney damage from $NaAsO_2$ exposure, offering potential therapeutic applications for arsenic toxicity management.

Keywords N. latifolia, Oxidative stress, Sodium arsenite, Inflammation, Hepatorenal toxicity, Stem bark extract

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Background

The broad presence and severe toxicity of arsenic from which a compound called arsenic acid is gotten, make it highly hazardous to both environmental and public health, with its ubiquitous distribution in almost all types of environmental media including water, air, and soil (Fatoki & Badmus, 2022). Inorganic arsenic (iAs) in contaminated groundwater is the main exposure route to this metalloid, particularly in areas with naturally high soil arsenic levels (Li et al., 2023). Inorganic arsenic, especially as arsenite and arsenate, is regarded as the most toxic and at low levels significantly affects kidney cell function (Fatoki & Badmus, 2022). Thus, the kidneys are a primary target for arsenic associated toxicity due to the severe retention of this inorganic metalloid. Exposure to arsenic disrupts mitochondrial function within kidney cells, leading to significant damage to renal tissues (Bongiovanni et al., 2019). In humans, the liver is critical in the metabolism and detoxification of arsenic; chronic arsenic exposure in humans causes liver damage, which may manifest as hepatocellular carcinoma. Arsenic and its metabolites tend to accumulate in the liver, which leads to oxidative stress, inflammation, and cell injury (Shao et al., 2024).

Research has documented the existence of medicinal plants possessing antioxidant and anti-inflammatory effects (Iheagwam et al., 2020). Among these plants, members of the extensive Rubiaceae family, comprising the genus *Nauclea*, which comprises approximately 35 species distributed globally, have garnered significant interest. *N. latifolia,* also known as the African peach, grows in Sub-Saharan Africa*.* This evergreen shrub or small tree is found on the edges of tropical forests and in wooded savannah. Local communities have historically employed *N. latifolia* in ethnomedicine, with reports of its long-standing use in Central and West Africa (Haudecoeur et al., 2018). In ethnomedicine, plants are used to treat a wide range of illnesses, such as malaria, infections, diabetes, and pain. Many secondary metabolites have been identified and studied, such as pentacyclic triterpenoids, monoterpene indoles, alkaloids, and several phenolic compounds (Boucherle et al., 2016; Haudecoeur et al., 2018). Experimentally, it has been shown that *N. latifolia* has a range of benefits including anti-inflammatory, antioxidant, anti-noceptive, hypoglycemic, and others (Haudecoeur et al., 2018). The study evaluated the protective effects of stembark aqueous extract of *N. latifolia* on the liver, and kidney from the harmful effects of arsenic acid. Adult Wistar rats were used to test the aqueous extract's anti-inflammatory, antioxidant, and anti-hyperlipidemic effects.

Methods

Chemicals and reagents

Sodium arsenite ($NaAsO_2$) (Sigma Aldrich Co., LLC, St. Louis, USA) was utilized to induce organ damage in the study. Silymarin, sourced from Micro Labs Ltd. in India, was acquired from a reputable manufacturer and served as the reference drug. Additional materials used in the study included 70% ethanol, phosphate-buffered saline (PBS), and ketamine hydrochloride as the anaesthetic. The levels of the biochemical markers used in the study were assessed using colorimetric diagnostic kits. Antioxidant enzyme activities bio-markers were measured with laboratory diagnostic kits (Randox Laboratories Ltd., County Antrim, UK)). Additionally, an ELISA kit (Koon Biotech Co., Ltd, China) was employed to measure inflammatory markers, including TNF-α and interleukin in rats.

Plant Material Collection and Identification

Plant Material

Fresh stem bark of *N. latifolia* (African peach) was collected from Shika in Gabon gari, Zaria, Northwest Region of Nigeria. The plant material was authenticated by a qualified botanist at Ahmadu Bello University (ABU), Zaria. A voucher specimen (number 3276): was deposited at the Department of Biological Sciences, (ABU), Zaria for future reference.

Sample Preparation and Extraction

The stem bark was cleaned, air-dried under shade for 14 days, and pulverized into fine powder using a mechanical grinder. The aqueous extract was prepared using a previously established methodology (Mbah et al., 2012). Briefly, 100 g of the powdered stem bark was soaked in 1000 ml of distilled water for 24 h, then boiled for 30 min. The mixture was filtered using Whatman No. 1 filter paper, and the filtrate was concentrated using a rotary evaporator at 40 °C. The concentrated extract was freeze-dried to obtain the final aqueous extract powder, which was stored at 4 °C until use.

Animals and ethical approval

The study followed the ARRIVE 2.0 guidelines for animal research. ABU's animal ethics committee approved the protocol, reference ABUCAUC/2023/045. A total of twenty-five (25) albino Wistar rats weighing between 140 and 160 g were selected for the experiment. The rats were sourced from the university's Experimental Laboratory. Sample size was calculated using G*Power 3.1.9.7 software. With an effect size of 0.90 (based on preliminary data showing large differences between treatment groups), α-error probability of 0.05, and

power of 0.80, the minimum required sample size was determined to be 4 animals per group. We used 5 animals per group to account for potential attrition and to ensure adequate statistical power. Polypropylene cages were used for rats with 12-h light/dark cycles, 45 ± 5% relative humidity, and a temperature of 24 ± 2 °C. They were provided with unlimited access to tap water and fed a pellet-based rat chow diet. The rats underwent a one-week acclimation period at the animal facility prior to the start of the experiments.

Experimental Design

Twenty-five adult male Wistar rats (200-250 g) were randomly divided into five groups (n = 5 per group):

Group I (NC): Received normal saline (1 ml/kg) orally for 28 days without any other treatment.

Group II (5 mg/kg $NaAsO_2$): Received sodium arsenite (5 mg/kg) intraperitoneally for 28 days.

Group III (200 mg/kg NLAE + $NaAsO_2$): Received *N. latifolia* stem bark aqueous extract (200 mg/kg) orally, followed by sodium arsenite (5 mg/kg) intraperitoneally, both for 28 days.

Group IV (400 mg/kg NLAE + $NaAsO_2$): Received *N. latifolia* stem bark aqueous extract (400 mg/kg) orally, followed by sodium arsenite (5 mg/kg) intraperitoneally, both for 28 days.

Group V (50 mg/kg SLY + $NaAsO_2$): Received silymarin (50 mg/kg) orally, followed by sodium arsenite (5 mg/kg) intraperitoneally, both for 28 days.

The doses of *N. latifolia* stem bark aqueous extract (200 and 400 mg/kg) were selected based on preliminary acute toxicity studies and previous phytochemical investigations. According to Kouadio et al., (2014), the LD_{50} of *N. latifolia* aqueous extract was determined to be > 5000 mg/kg in rats, indicating low toxicity. The selected doses represent 1/25 and 1/12.5 of the LD_{50} value, respectively, ensuring safety while providing therapeutic efficacy. These doses have been previously shown to exhibit significant antioxidant activity in similar experimental models (Gidado et al., 2008). The sodium arsenite dose (5 mg/kg) was selected based on previous studies demonstrating significant hepatorenal toxicity without causing mortality (Adil et al., 2015; Sharma et al., 2021). Silymarin (50 mg/kg) was used as a positive control based on its established hepatoprotective properties (Kumar & Khanna, 2018).

Animal sacrifice, collection, and preparation of samples

All animals in each group were weighed both at the start and conclusion of the experiment. The rats received an intramuscular injection of Ketamine Hydrochloride in the amount of 50 mg/kg. We obtained blood samples via cardiac puncture, transferred into sterile expository bottles and centrifuged at 5000 g for a period of 10 min. The clear serum that was obtained was drawn carefully with an automatic pipette and decanted into fresh sterile tubes for examination.

Estimation of liver enzymes and kidney parameters

The evaluation of serum biomarkers of liver injury was carried out using commercial diagnostic kits of Randox Laboratories Ltd., UK. Alkaline phosphatase (ALP) activity was defined according to the modified protocols designed by Wright et al. (1972) and alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were measured as per the methods of Reitman and Frankel (1957) with technical modifications. The concentration of serum total protein and albumin was measured with Bjorston et al. (2007) procedures, and supplementary protocols were used to estimate the total protein and calculate the concentration of albumin Doumas et al. (1971) and Rengarajan et al. (1989). Randox Laboratories kits were employed to analyses the kidney function biomarkers strictly as per the manufacturer instructions (Patton & Crouch, 1977).

Determination of oxidative stress and pro-inflammatory cytokines

For biochemical analysis, the collected organs were initially weighed and then homogenised (liver for antioxidant indicators) in a 50 mmol/l Tris–HCl buffer with a pH of 7.4. After homogenisation, the homogenate was centrifuged for 15 min at 5000 g. The supernatants obtained were promptly frozen and stored for later use. Oxidative stress markers (SOD, CAT, GSH) were measured using commercially available Randox kits (Randox Laboratories Ltd., County Antrim, UK). The Randox SOD kit (Cat. No. SD125) utilizes the xanthine oxidase method with a detection range of 0–300 U/ml. The Randox CAT kit (Cat. No. CA515) employs the hydrogen peroxide substrate method with a sensitivity of 0.1 U/ml. The Randox GSH kit (Cat. No. GR2368) uses the DTNB recycling method with a measurement range of 0–100 μmol/L. All assays were performed according to manufacturer's instructions using a semi-automated analyzer (Randox RX series).

Pro-inflammatory cytokines (TNF-α and IL-6) were measured using enzyme-linked immunosorbent assay (ELISA) kits. TNF-α levels were determined using the Rat TNF-α. ELISA Kit (Cat. No. YX-C-A511, Koon Biotech Co., Ltd, China) with a sensitivity of 5.0 pg/ml and detection range of 15.6–1000 pg/ml. IL-6 levels were measured using the Rat IL-6 ELISA Kit (Cat. No. YX-C-A516, Koon Biotech Co., Ltd, China) with a sensitivity of 21.0 pg/ml and detection range of 62.5–4000 pg/ml. All

procedures were performed according to manufacturer's protocols.

Haematoxylins and eosin staining

Tissues were preserved in formalin to maintain cyto-architecture and prevent degeneration of the tissue. The samples were then dehydrated using a series of alcohol baths with progressively increasing concentrations to eliminate water. A clearing agent (xylene) was applied to render the tissues transparent, allowing for the infiltration of paraffin wax. The tissues were subsequently embedded in molten paraffin wax, which replaced the clearing agent. The infiltrated tissue is cooled and solidified in a paraffin block for sectioning. Thin sections (typically 4–5 µm) were cut using a microtome. Sections are stained first with haematoxylin, which binds to nucleic acids, followed by eosin, which stains cytoplasmic components (Feldman & Wolfe, 2014).

Statistical analysis

The data obtained were presented as the mean ± SEM from several experiments (n = 5). One-way ANOVA followed by Tukey's post hoc test was used in SPSS 21.0 (SPSS, Cary, NC, USA). When the p-value is less than 0.05, it is considered significant. The ANOVA is used to determine if three or more independent groups differ statistically significantly. It helps in assessing whether any of the variations observed in the data are due to the manipulation of an experimental factor rather than chance. Multiple groups in a controlled experiment can benefit from this analysis.

Results

Effects of N. latifolia on the weights of rats exposed to sodium arsenite

Rats treated with $NaAsO_2$ demonstrated a relatively lesser weight gain ($P < 0.05$) when compared with the NC group and the treated 200/400 mg/kg NLAE + $NaAsO_2$ groups. However, the treated groups of 200/400 mg/kg NLAE + $NaAsO_2$ had significant weight gains in comparison to the $NaAsO_2$ group. Also, there was a significant difference ($P < 0.05$) between the organ weight of the rats administered with only $NaAsO_2$ and those in the saline control or treatment groups 200/400 mg/kg NLAE + $NaAsO_2$ and 50 mg/kg SLY + $NaAsO_2$. Gonadal-weight loss was noted even in 200/400 mg/kg NLAE + $NaAsO_2$ or SLY + $NaAsO_2$ treated rats in contrast to $NaAsO_2$ only rats. In contrast however, somatic indices between the treated and control groups did not appear to fluctuate ($p > 0.05$). $NaAsO_2$ treated rats had significantly reduced testis somatic indices compared to the control, and 200 or 400 mg/kg NLAE + $NaAsO_2$ or 50 mg/kg SLY + $NaAsO_2$ rats (Table 1).

Effects of N. latifolia on liver enzyme renal function parameter in rats exposed to sodium arsenite

In the groups receiving 200/400 mg/kg NLAE + $NaAsO_2$, serum AST, ALP, and ALT levels showed a significant decrease ($p < 0.05$) compared to the $NaAsO_2$ group. In contrast, the $NaAsO_2$ group exhibited a notable increase ($p < 0.05$) in these serum markers when compared to the control group, the 200/400 mg/kg NLAE + $NaAsO_2$ groups, and the 50 mg/kg SLY + $NaAsO_2$ group. Animals which were exposed to treatment with 200/400 mg/kg NLAE + $NaAsO_2$ increased total protein and albumin levels as compared to rats treated with $NaAsO_2$. While sodium arsenate treated rats however had significantly lower levels of serum protein and albumin ($p < 0.0001$) compared to the NC group. There was no statistically significant variance in serum protein level as well, in rats receiving SLY 50 mg/kg + $NaAsO_2$ when compared to levels in the $NaAsO_2$ group.

All the kidney markers levels were significantly elevated ($P < 0.001$) in the $NaAsO_2$ group compared to

Table 1 Protective Effect of *N. latifolia* on Body and organ weight of the adult male Wistar Rats-induced Arsenic Acid Toxicity

Parameters	Control (Mean ± S.E)	$NaAsO_2$ (Mean ± S.E)	200 mg/kg NLAE (Mean ± S.E)	400 mg/kg NLAE (Mean ± S.E)	50 mg/kg SLY (Mean ± S.E)	F	*P-Value*
Initial weight (g)	148.60 ± 4.55	159.00 ± 3.59	152.20 ± 2.85	156.80 ± 1.83	152.00 ± 2.66	1.647	0.202
Final weight (g)	204.80 ± 2.93^{c}	178.00 ± 5.03^{a}	175.00 ± 5.03^{a}	201.60 ± 5.07^{bc}	182.80 ± 8.55^{ab}	7.329	< 0.001
Weight gain (g)	60.20 ± 1.98^{c}	19.00 ± 3.59^{a}	23.20 ± 2.52^{a}	44.80 ± 3.59^{bc}	30.80 ± 6.82^{ab}	17.329	< 0.001
Liver weight (g)	5.20 ± 0.11^{a}	6.86 ± 0.17^{c}	6.08 ± 0.22^{b}	5.72 ± 0.09^{ab}	6.04 ± 0.24^{a}	17.272	< 0.001
Kidney weight (g)	0.58 ± 0.04	0.76 ± 0.09	0.49 ± 0.04	0.60 ± 0.06	0.78 ± 0.13	11.545	0.001
Rel. Liver weight	2.49 ± 0.05^{a}	3.87 ± 0.18^{d}	3.48 ± 0.17^{cd}	2.84 ± 0.04^{ab}	3.31 ± 0.12^{bc}	5.321	< 0.004
Rel. Kidney weight	0.27 ± 0.02^{a}	0.43 ± 0.05^{b}	0.30 ± 0.02^{a}	0.31 ± 0.03^{a}	0.35 ± 0.07^{ab}	18.659	< 0.001

Table 1 shows the effects of various treatments on body weight as well as the weights of of the liver, and kidneys. Control (NC) rats were treated with just distilled water. $NaAsO_2$: rats intoxicated with sodium arsenite; 200/400 NLAE + $NaAsO_2$: rats treated with NLAE and sodium arsenite intoxication; SLY + $NaAsO_2$: rats treated with silymarin and sodium arsenite. Values in the same row with different superscripts ($^{a, b}$, and ^{c}) indicate a significant difference (p < 0.001) n = 5

the control. However, the levels of these renal markers were significantly lower ($p < 0.05$) in the rats treated with 200/400 mg/kg NLAE + $NaAsO_2$ than those of the $NaAsO_2$ group. Despite this, the 200/400 mg/kg NLAE + $NaAsO_2$ groups continued to show higher concentrations of urea, uric acid and creatinine than the control groups. Compared to the $NaAsO_2$ group, SLY + $NaAsO_2$ treated rats showed lower levels of renal biomarkers. No significant differences ($p > 0.05$) were observed in renal bio-makers levels between the NLAE + $NaAsO_2$ group and the 50 mg/kg SLY + $NaAsO_2$ group. (Table 2).

Effects of N. latifolia on oxidative stress of rats exposed to sodium arsenite

$NaAsO_2$-treated rats had lower GSH, SOD, and CAT enzyme activities than NC rats. Also, the level of CAT, level of GSH, and level of SOD were significantly higher ($p < 0.001$) compared to the $NaAsO_2$ group in the 200 and 400 mg/kg NLAE + $NaAsO_2$ and SLY + $NaAsO_2$ treated groups. There was no significant difference ($p > 0.05$) with regards to CAT levels between the 200 mg/kg NLAE + $NaAsO_2$ group and the $NaAsO_2$ treated group (Fig. 3. A-C). Compared to NC, $NaAsO_2$ had significantly higher MDA levels, and they were significantly low ($P < 0.05$) with the 400 mg/kg NLAE + $NaAsO_2$ and SLY + $NaAsO_2$ treated groups compared to the $NaAsO_2$ group (Fig. 1A-D).

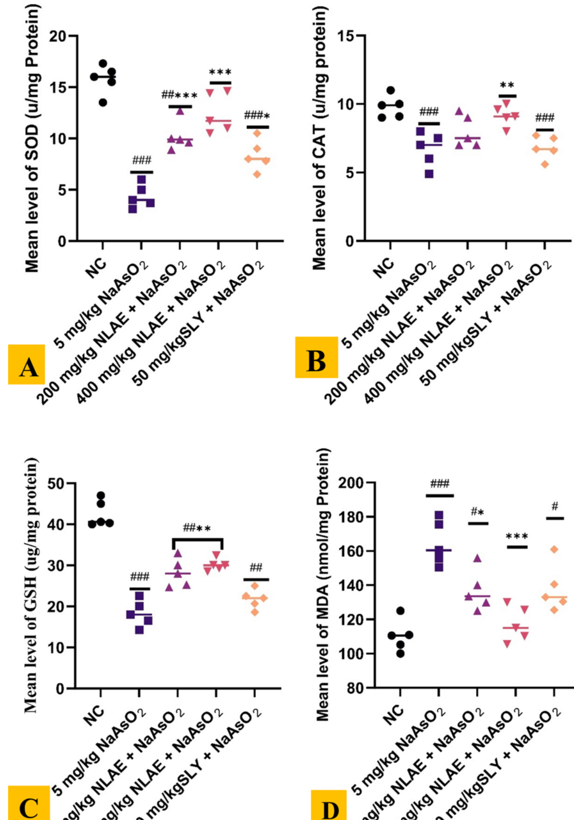

Fig. 1 Bar charts of Inflammatory markers (**A**) SOD, (**B**) CAT, (**C**) GSH (**D**) MDA. *Significant difference compared with $NaAsO_2$ control *$P < 0.05$; **$P < 0.002$; ***$P < 0.0001$. #Significant difference compared with control (NC) #$P < 0.05$; ##$P < 0.002$; ###$P < 0.0001$. n = 5

Effect of N. latifolia on IL-6 and TNF-α of rats exposed to sodium arsenite

The mean concentrations of TNF-α and IL-6 are illustrated in Fig. 1. The $NaAsO_2$ treatment group had a higher concentration of TNF-α and IL-6 as compared to the NC group ($P < 0.05$). These levels were however significantly lower in the 200/400 mg/kg NLAE + $NaAsO_2$ and SLY + $NaAsO_2$ groups as compared to the $NaAsO_2$ group. A significant difference did not exist ($P > 0.05$) between the 200/400 mg/kg NLAE + $NaAsO_2$ and SLY + $NaAsO_2$ treatment groups (TNF-α concentration) (Fig. 2A & B).

Table 2 Protective Effect of *N. latifolia* on the Liver enzymes and Kidney Function Biomarkers of the Adult Male Wistar Rats-induced Arsenic Acid Toxicity

Parameters	Control (Mean ± S.E)	$NaAsO_2$ (Mean ± S.E)	200 mg/kg NLAE (Mean ± S.E)	400 mg/kg NLAE (Mean ± S.E)	50 mg/kg SLY (Mean ± S.E)	F	*P-Value*
ALT (IU/L)	9.80 ± 1.49^{a}	28.00 ± 0.95^{d}	17.20 ± 1.46^{bc}	12.40 ± 0.51^{ab}	19.20 ± 1.88^{c}	27.308	< 0.001
AST (IU/L)	26.40 ± 1.86^{a}	71.40 ± 2.11^{d}	51.20 ± 4.14^{c}	39.40 ± 2.13^{b}	45.60 ± 1.96^{b}	41.075	< 0.001
ALP (IU/L)	24.42 ± 0.89^{a}	58.90 ± 1.12^{c}	42.40 ± 1.01^{b}	30.74 ± 1.71^{a}	40.16 ± 2.82^{b}	61.665	< 0.001
Total Protein (mg/dL)	14.94 ± 0.71^{b}	9.62 ± 1.86^{a}	11.58 ± 0.52^{ab}	12.68 ± 0.42^{ab}	12.24 ± 1.23^{ab}	4.786	0.007
Albumin (mg/dL)	3.64 ± 0.21^{c}	1.96 ± 0.27^{a}	2.52 ± 0.26^{ab}	3.16 ± 0.26^{bc}	2.40 ± 0.18^{ab}	7.498	< 0.001
Urea (mg/dL)	45.60 ± 2.09^{a}	76.70 ± 4.17^{b}	52.00 ± 3.56^{a}	48.01 ± 1.76^{a}	54.23 ± 2.98^{a}	16.628	< 0.001
Creatinine (meq/L)	0.88 ± 0.05^{a}	1.54 ± 0.09^{b}	1.16 ± 0.08^{ab}	1.06 ± 0.09^{a}	1.22 ± 0.12^{ab}	7.256	< 0.001
Uric Acid	10.98 ± 0.79^{a}	17.70 ± 0.46^{c}	10.60 ± 0.51^{b}	9.40 ± 0.63^{b}	11.00 ± 0.55^{b}	64.225	< 0.001

Table 2 shows the effects of various treatments on liver enzymes and kidney function parameters after 28 days of the experiment. Values in the same row with different superscripts ([a], [b], and [c]) indicate a significant difference ($p < 0.001$). n = 5

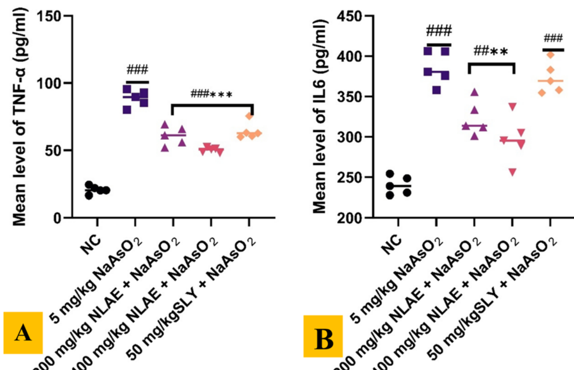

Fig. 2 Bar charts of Inflammatory markers (A) TNF-α (B) IL-6. TNF-α = , Tumour Necrosis Factor Alpha.; IL-6 = interleukin -6. *Significant difference compared with $NaAsO_2$ control *P < 0.05; **P < 0.002; ***P < 0.0001. #Significant difference compared with control (NC) #P < 0.05; ##P < 0.002; ###P < 0.0001. n = 5

Effects of N. latifolia on the histology of rats exposed to sodium arsenite

A healthy control rat had normal liver sinusoids, hepatocytes, and central veins (Fig. 3A). Rat livers treated with $NaAsO_2$ showed evidence of steatosis, fat hepatocellular vacuoles, and hepatocyte degeneration (Fig. 3B). Hepatocellular vacuoles containing liver fat were negligible in rats treated with 200/400 mg/kg NLAE + $NaAsO_2$ (Fig. 3C & D). Administration of SLY and $NaAsO_2$ led to significant sinusoid expansion and minimal vacuolation of hepatocytes (Fig. 2E). The kidneys of normal control rats exhibit typical histological structures, characterised by typical tubular structures and glomeruli (Fig. 4A). Rat kidneys exposed to $NaAsO_2$ showed severe glomerulus retraction and localized degeneration (Fig. 4B). Rats given 200/400 mg/kg NLEA along with $NaAsO_2$ had kidneys with little sinusoid dilation and slightly damaged glomeruli (Fig. 4C and D). Rats administered SLY and $NaAsO_2$ likewise displayed notable glomerular retraction and degeneration (Fig. 4E).

Discussion

The current research tested the protective effects of NLAE against $NaAsO_2$-induced hepatorenal oxidative stress, inflammation, and histopathological changes in Wistar rats. We find that exposure to $NaAsO_2$ resulted in hepatic and renal injury, which is characterized by increased serum liver enzymes, kidney functions biomarkers, and oxidative stress, pro-inflammatory cytokines, and histological degradation. These negative effects were significantly reduced by pretreatment with NLAE at 200 and 400 mg/kg and silymarin, a reference drug, which indicates that *N. latifolia* has strong antioxidant and anti-inflammatory properties.

$NaAsO_2$-treated rats showed severe loss of weight and relative liver and kidney weight gain relative to the control (Table 1). The changes are in line with the earlier reports of arsenic exposures causing altered body metabolism and organ hypertrophy following inflammation and oxidative stress (Lima et al., 2018; Rahaman et al., 2021). NLAE treatment, especially with 400 mg/

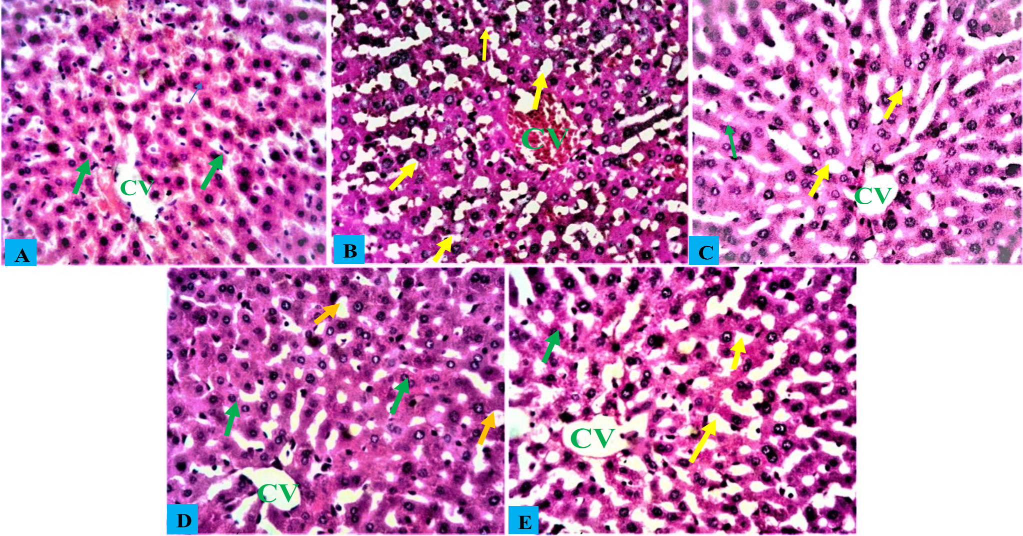

Fig. 3 The control liver photomicrograph (**A**) showed normal hepatocytes and central vein, whereas the photomicrograph from the $NaAsO_2$-treated group (**B**) showed deteriorating hepatocytes and fat-filled hepatocellular vacuoles (yellow arrow). NLAE 200/400 mg/kg + 5 mg/kg $NaAsO_2$ (**C**&**D**) showed mildly vacuolated hepatocytes (yellow arrow) and normal hepatocytes (green arrow); Silymarin + 5 mg/kg $NaAsO_2$-treated rats (**E**) showed dilated sinusoids and vacuolated hepatocytes (blue arrow) H and E staining at X200 magnification

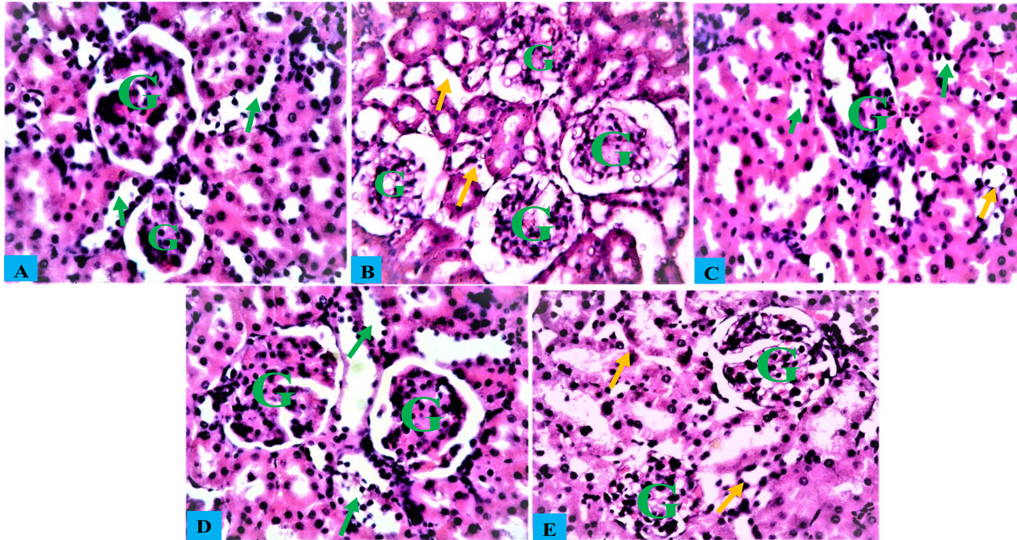

Fig. 4 The control kidney photomicrograph (**A**) displayed a normal glomerulus (G) and renal tubules. The micrograph from the NaAsO2-treated group (**B**) revealed severe spontaneous lipid vacuolation and a shrunken glomerulus (G). The group treated with NLAE 200/400 mg/kg plus 5 mg/kg $NaAsO_2$ showed a mildly obliterative glomerular space (**C**) along with normal renal tubules and a normal glomerulus (**D**). In the Silymarin plus 5 mg/kg $NaAsO_2$-treated rats (**E**), an obliterative glomerulus form with spontaneous lipid vacuolation (G) was observed. H and E staining at X200 magnification

kg, also reversed weight gain and restored the weights of organs indicating the protective effect of NLAE on the metabolism of the entire system and the integrity of organs. Busari et al. (2021) also made similar observations in which *N. latifolia* reversed the toxin-induced hepatic and renal weight changes using its bioactive phytochemicals.

$NaAsO_2$ dramatically increased serum ALT, AST, and ALP activity and reduced total protein and albumin concentrations (Table 2), which suggested the hepatocellular leakage and synthetic dysfunction. Higher levels of transaminases are made to be the indexes of necrosis and oxidative damage of the liver caused by arsenic (Muthumani & Miltonprabu, 2015). Conversely, rats in the NLAE-treated group registered significantly decreased liver enzyme levels and regained protein and albumin levels, and the 400 mg/kg dose exhibited similar effects to silymarin. These results support the previous research showing the hepatoprotective effects of *N. latifolia* through free radical scavenging and membrane stabilizing (Ayeleso et al., 2014; Busari et al., 2021). The level of serum urea, creatinine, and uric acid in rats exposed to $NaAsO_2$ were very high and confirmed the presence of nephrotoxicity as had been previously reported in arsenic toxicity models (Xu et al., 2020; Zheng et al., 2014). These renal biomarkers were greatly decreased by treatment with NLAE, and glomerular filtration and tubular reabsorption were functionally recovered. This effect of the plant is probably the result of the polyphenolic compounds that decrease the oxidative stress damage to renal tissues (Iheagwam et al., 2020).

$NaAsO_2$ intoxication translated into considerable depletion of antioxidant enzymes SOD, CAT and GSH, with increased MDA levels, a lipid peroxidation product (Fig. 1). Such an oxidative imbalance is in line with findings that attribute arsenic to over production of ROS and over-depletion of antioxidants (Das et al., 2010; Flora et al., 2007). Surprisingly, NLAE treatment reverted the SOD, CAT, and GSH levels in addition to decreasing the levels of MDA, and showed strong antioxidative properties similar to silymarin. Enhancement in antioxidative activity by *N. latifolia* in toxin-induced hepatic and renal injuries was previously reported (Ayeleso et al., 2014; Busari et al., 2021). Pro-inflammatory cytokines TNF-α and IL-6 were significantly elevated in $NaAsO_2$-treated rats, reflecting arsenic-induced inflammatory cascades (Singh et al., 2015; Zhu et al., 2014). Treatment with NLAE markedly suppressed TNF-α and IL-6 levels (Fig. 2), indicating inhibition of oxidative stress-mediated inflammatory pathways. These results agree with Iheagwam et al. (2020), who demonstrated the anti-inflammatory potential of *N. latifolia* extracts in experimental models.

Histological examination showed acute hepatocellular steatosis, vacuolar, and necrosis, and renal glomerular atrophy and tubular necrosis in $NaAsO_2$-treated rats (Figs. 3 and 4). Such structural changes are associated

with the increased levels of biochemical indicators and cytokines, which prove the oxidative and inflammatory tissue damage (Singh et al., 2011; Zheng et al., 2014). Interestingly, hepatic and renal cytoarchitecture was preserved in NLAE-treated groups with little vacuolation, which supports structural recovery in line with biochemical evidence. Busari et al. (2021) report similar histological protection of N. latifolia against injury caused by toxins to organs. The suggested mechanism of *N. latifolia*-hepatorenal protection is likely due to its antioxidative, anti-inflammatory, and membrane-stabilizing phytochemicals (alakoid, flavonoid, and phenolics) (Boucherle et al., 2016; Haudecoeur et al., 2018). *N. latifolia* is also likely to break the oxidative-inflammatory cascade of tissue injury caused by arsenic by increasing the activity of endogenous antioxidants (SOD, CAT, GSH) and inhibiting lipid peroxidation and release of cytokines.

The study suggests that *N. latifolia* aqueous extract could provide an affordable, plant-based therapeutic option for arsenic toxicity, especially in areas with high exposure and limited access to conventional treatments. Its similar efficacy to silymarin highlights its potential as an alternative or complementary therapy. Limitations to the study are the absence of an extract-only control group to establish baseline effects of *N. latifolia*, the evaluation at a single time point which restricts understanding of changes over time, and a lack of mechanistic examination of the pathways; hence, future studies should concentrate on detailed mechanistic investigation, phytochemical characterization of bioactive compounds, well-designed clinical trials to verify safety and efficacy, and evaluation of combination therapies with other protective agents.

Conclusion

This study shows that *N. latifolia* stem bark aqueous extract offers dose-dependent protection against sodium arsenite-induced liver and kidney damage in Wistar rats by restoring antioxidant enzyme activity, lowering inflammatory markers, and preserving tissue structure. Its efficacy, comparable to silymarin, highlights its potential as a natural therapeutic option for arsenic toxicity. Further research is needed to clarify its mechanisms, identify active compounds, and confirm safety for clinical use, supporting plant-based interventions in arsenic-affected regions.

Abbreviations

$NaAsO_2$	Sodium arsenite
NLAE	*Nauclea latifolia* aqueous extract
SLY	Silymarin

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Author contributions

Conceptualization: WM, VKJ, GKA. Data acquisition: WM, OBO, AA. Data analysis or interpretation: WM, VKJ, GKA. Drafting of the manuscript: WM, BI, AYS. Critical revision of the manuscript: WM, AA, OBO. Approval of the final version of the manuscript: all authors.

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Data availability

The raw data supporting the findings of this study will be readily made available upon request from the corresponding author.

Declarations

Ethics and participation consent

This research was conducted according to the requirements of the ARRIVE Guidelines and has been approved by local animal ethics committee of ABU, Zaria, Nigeria (ABUCAUC/2023/045).

Consent for publication

Not Applicable.

Competing interests

The authors declare no competing interests.

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